ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

ARCHIVIO ISTITUZIONALE
DELLA RICERCA

Alma Mater Studiorum Università di Bologna
Archivio istituzionale della ricerca

An in vitro evaluation of the effects of a Yucca schidigera extract and chestnut tannins on composition and metabolic profiles of canine and feline faecal microbiota

This is the final peer-reviewed author’s accepted manuscript (postprint) of the following publication:

Published Version:

Availability:
This version is available at: https://hdl.handle.net/11585/605587 since: 2020-01-09

Published:

DOI: http://doi.org/10.1080/1745039X.2017.1355039

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/).
When citing, please refer to the published version.

(Article begins on next page)

19 April 2024

This is an Accepted Manuscript of an article published by Taylor & Francis in Archives of Animal Nutrition on 31 Jul 2017, available online: http://www.tandfonline.com/ https://doi.org/10.1080/1745039X.2017.1355039

Without prejudice to other rights expressly allowed by the copyright holders, this publication can be read, saved and printed for research, teaching and private study. Any other noncommercial and commercial uses are forbidden without the written permission of the copyright holders.

24 **_An in vitro_ evaluation of the effects of a _Yucca schidigera_ extract and chestnut**
25 **tannins on composition and metabolic profiles of canine and feline faecal**
26 **microbiota**
27

28 Carlo Pinna[a,], Carla Giuditta Vecchiato[a], Vladimiro Cardenia[b], Maria Teresa
29 Rodriguez-Estrada[c], Claudio Stefanelli[d], Monica Grandi[a], Pier Paolo Gatta[a],
30 Giacomo Biagi[a]
31
32 [a]Department of Veterinary Medical Sciences, University of Bologna, Via Tolara di
33 Sopra 50, 40064 Ozzano Emilia, Italy; [b]Interdepartmental Centre for Agri-Food
34 Industrial Research, University of Bologna, Via Quinto Bucci 336, 47521 Cesena,
35 Italy; [c]Department of Agricultural and Food Sciences, University of Bologna, Viale
36 Fanin 40, 40127 Bologna, Italy; [d]Department for Life Quality Studies, University of
37 Bologna, Corso d'Augusto 237, 47921 Rimini, Italy
38
39 Corresponding author: Dr. Carlo Pinna, Department of Veterinary Medical Sciences,
40 University of Bologna, Via Tolara di Sopra 50, 40064 Ozzano dell'Emilia, Italy - Tel:
41 +39 0512097383; Fax: +39 0512097373; Email: carlo.pinna2@unibo.it
42 Carla Giuditta Vecchiato DVM, Department of Veterinary Medical Sciences,
43 University of Bologna, Via Tolara di Sopra 50, 40064 Ozzano dell'Emilia (BO),
44 Italy - Tel. +39 0512097383 - Fax +39 051 2097373 - Email:
45 carla.vecchiato2@unibo.it
46 Dr. Vladimiro Cardenia, Interdepartmental Centre for Agri-Food Industrial Research,
47 University of Bologna, Via Quinto Bucci 336, 47521 Cesena, Italy – Tel. +39
48 0512096015

49 Dr. Maria Teresa Rodriguez-Estrada, Department of Agricultural and Food Sciences,
50 University of Bologna, Viale Fanin 40, 40127 Bologna, Italy - Tel. +39 051 2096011
51 - Fax +39 0512096017 - Email: maria.rodriguez@unibo.it
52 Prof. Claudio Stefanelli, Department for Life Quality Studies, University of Bologna,
53 Corso d'Augusto 237, 47921 Rimini, Italy – Tel. +39 0512091207 – Fax +39
54 0512091224
55 Dr. Monica Grandi, Department of Veterinary Medical Sciences, University of
56 Bologna, Via Tolara di Sopra 50, 40064 Ozzano dell'Emilia (BO), Italy - Tel.
57 +39.051.2097383 - Fax +39.051.2097373 - Email: monica.grandi8@unibo.it
58 Prof. Pier Paolo Gatta, Department of Veterinary Medical Sciences, University of
59 Bologna, Via Tolara di Sopra 50, 40064 Ozzano dell'Emilia (BO), Italy - Tel.
60 +39.051.2097032 - Fax +39.051.2097373 - Email: pierpaolo.gatta@unibo.it
61 Prof. Giacomo Biagi, Department of Veterinary Medical Sciences, University of
62 Bologna, Via Tolara di Sopra 50, 40064 Ozzano dell'Emilia (BO), Italy - Tel.
63 +39.051.2097379 - Fax +39.051.2097373 - Email: giacomo.biagi@unibo.it
64

65 **Abstract**

66 The *in vitro* effect of a *Yucca schidigera* extract and tannins from chestnut wood on
67 composition and metabolic activity of canine and feline faecal microbiota was
68 evaluated. Four treatments were carried out: control diet (Group CTRL), chestnut
69 tannins (Group CT), *Y. schidigera* extract (Group YSE) and Group CT+YSE. The YSE
70 was added to canine and feline faecal cultures at 0.1 g/l, while CT were added at 0.3
71 g/l for a 24 h incubation. A total of 130 volatile compounds were detected by means
72 of HS-SPME-GC/MS analyses. Several changes in the metabolite profiles of
73 fermentation fluids were found including a decrease of alcohols (- 19%) and esters (-
74 42%) in feline and canine inoculum, respectively, due to the antibacterial properties of

75 tannins. In canine inoculum, after 6 h, Group YSE+CT resulted in lower cadaverine
76 concentrations (- 37%), while ammonia (- 4%) and quinolone (- 27%) were reduced
77 by Group CT. After 24 h, the presence of CT resulted in a decrease of sulphur
78 compounds, such as dimethyl sulphide (- 69%) and dimethyl disulphide (- 20%). In
79 feline faecal cultures, after 6 h, Group CT lowered the amount of indole (- 48%),
80 whereas Group YSE tended to decrease trimethylamine levels (- 16%). Both in canine
81 and feline inoculum, Group CT and, to a minor extent, Group YSE affected volatile
82 fatty acids patterns. In canine faecal cultures, tannins exerted an inhibitory effect on
83 *E. coli* population (- 0.45 log10 numbers of DNA copies/ml), while enterococci were
84 increased (+ 2.06 log10 numbers of DNA copies/ml) by Group YSE. The results from
85 the present study show that *Y. schidigera* extract and tannins from chestnut wood exert
86 different effects on the composition and metabolism of canine and feline faecal
87 microbiota. In particular, the supplementation of *Y. schidigera* and tannins to diets for
88 dogs and cats may be beneficial due to the reduction of some potentially toxic volatile
89 metabolites.
90

91 *Keywords:* dogs; cats; intestinal microorganisms; *Yucca schidigera*; tannins; volatile
92 compounds.
93

94 *Abbreviations:* ADF, acid detergent fibre; ADL, acid detergent lignin; CT, tannins
95 from chestnut wood; DM, dry matter; FOS, fructo-oligosaccharides; GC/MS, gas
96 chromatography–mass spectrometry; NDF, neutral detergent fibre; PCA, principal
97 components analysis; HS-SPME, headspace solid-phase microextraction; VFA,
98 volatile fatty acids; VOCs, volatile organic compounds; YSE, *Yucca schidigera*
99 extract.
100

101 ## 1. Introduction

102 The canine and feline gastrointestinal tract is inhabited by a variety of complex
103 microbial communities that play a fundamental role in maintaining the nutritional and
104 health status of the host. Diet is recognized as one of the major factors driving the
105 composition and metabolism of the gut microbiota (Russell et al. 2013). While many
106 studies have highlighted the beneficial effects deriving from the dietary inclusion of
107 non-digestible carbohydrates and prebiotics in dogs and cats (Pinna and Biagi 2014;
108 Rochus et al. 2014), little is known about feeding functional food components to these
109 animals.

110 *Yucca schidigera* Roezl ex Ortgies and tannin extracts are naturally occurring
111 plant substances that have been widely investigated in swine and poultry production
112 as potential phytochemical compounds for reducing odour and ammonia emissions
113 (Windisch et al. 2008; Biagi et al. 2010).

114 *Y. schidigera* is known to have antimicrobial (Wang et al. 2000), antiprotozoal
115 (McAllister et al. 2001), and antifungal (Miyakoshi et al. 2000) activities. The
116 biological effects of *Y. schidigera* have been attributed to its high content of steroidal
117 saponin fraction (Patra and Saxena 2009); however, Duffy et al. (2001) reported that
118 the observed effects were also due to the non-saponin fraction.

119 Tannins are natural polyphenolic compounds that can be chemically divided
120 into condensed and hydrolyzable tannins, whose structural diversity influences their
121 metabolism and bioavailability (Aura 2008). Tannins extracted from chestnut
122 (*Castanea sativa* Miller) wood are characterized by the presence of hydrolyzable
123 tannins, which are known to act as effective microbiota modulatory agents in poultry
124 (Jamroz et al. 2009), swine (Biagi et al. 2010), and ruminants (Hassanat and Benchaar
125 2013). While the administration of *Y. schidigera* to dogs and cats resulted in lower
126 faecal odour (Lowe et al. 1997; Lowe and Kershaw 1997; Giffard et al. 2001), to the

127 best of our knowledge, there are no studies on the influence of tannins on the canine
128 and feline intestinal ecosystems.

129 Considering that very little is known about the effects of *Y. schidigera* and
130 tannin extracts in dogs and cats, the aim of the present study was to evaluate *in vitro*
131 the effect of these extracts on the composition and activity of canine and feline faecal
132 microbiota. We hypothesized that adding *Y. schidigera* and tannin to canine and feline
133 faecal inocula may reduce the presence of potential pathogenic bacteria and toxic
134 compounds.

135

136 ## 2. Material and methods

137 The current study was carried out at the Laboratory of Animal Production of the
138 Department of Veterinary Medical Sciences, University of Bologna, Italy.

139

140 ### *2.1 Canine experiment*

141 Five healthy adult dogs (mixed breed; average BW 18.0 kg; age 4 to 6 year) were fed
142 the same commercial dry diet for adult dogs (Effeffe Pet Food S.p.A., Pieve di Porto
143 Morone, Italy), for 4 week before collection of fresh faecal samples. The diet contained
144 the following ingredients: dried poultry meat, rice and other cereals, oils and fat, dried
145 eggs by-products, vitamins, and minerals. The macronutrient composition of the diet
146 (as fed) was the following: water 53 g/kg, crude protein 229 g/kg, ether extract 154
147 g/kg, crude ash 69 g/kg, starch 370 g/kg, crude fibre 16 g/kg, neutral detergent fibre
148 (NDF) 137 g/kg, acid detergent fibre (ADF) 90 g/kg, and acid detergent lignin (ADL)
149 43 g/kg. A sample of fresh faeces was collected from each dog immediately after
150 excretion, pooled, and suspended at 10 g/l in prereduced Wilkins Chalgren anaerobe
151 broth. The faecal suspension was used to inoculate (100 ml/l) a previously warmed (39
152 °C) and prereduced medium prepared according to Sunvold et al. (1995). Five 30-ml

153 bottles (each bottle containing 21 ml of faecal culture) were set up per treatment. The
154 same dry food that was fed to the dogs used as faecal donors, was digested in triplicate
155 using the 2-step procedure proposed by Biagi et al. (2016). After *in vitro* digestion, the
156 undigested fraction was dried at 70 °C until a constant dry weight was obtained (19.3
157 g of undigested residue were obtained from 100 g of food dry matter (DM)) and its
158 chemical composition was the following: crude protein 169 g/kg, ether extract 100
159 g/kg, starch 17 g/kg, crude ash 238 g/kg, crude fibre 76 g/kg, NDF 313 g/kg, ADF 162
160 g/kg, and ADL 59 g/kg.

161 Four treatments were carried out: 1) control diet (CTRL) with no addition of
162 substrates, 2) group *Yucca schidigera* extract (group YSE), Syntonise (saponins
163 content 10.5%, Sintofarm S.p.A., Guastalla, Italy), 3) group chestnut tannins (group
164 CT), Farmatan 75 (tannins from chestnut wood, tannic acids 75.6%, Sintofarm S.p.A.,
165 Guastalla, Italy), 4) group YSE+CT. The YSE was added at a final concentration of
166 0.1 g/l, while CT was added at a final concentration of 0.3 g/l. The bottles also
167 contained the *in vitro* digested commercial dry food for dogs at 20 g/l. These
168 concentrations should reflect the amount of *Y. schidigera* and tannins extracts that
169 reach the hindgut when they are included in a commercial extruded food for dogs (with
170 a digestibility of approximately 80%) at a concentration of 1.0 and 3.0 g/kg,
171 respectively. In each study, five bottles were prepared without any experimental
172 substrate and with no addition of the digested food as a negative control. The pH of
173 faecal cultures was adjusted to 6.7; bottles were sealed and incubated for 24 h at 39 °C
174 in an anaerobic cabinet (Anaerobic System; Forma Scientific Co., Marietta, OH; under
175 an 85% N_2, 10% CO_2, and 5% H_2 atmosphere). Samples of fermentation fluid were
176 collected from each bottle at 6 and 24 h for the determination of pH, ammonia,
177 biogenic amines, volatile fatty acids (VFA), volatile compounds (VOCs), and for
178 microbial analysis.

179 ***2.2 Feline experiment***

180 Four healthy European shorthair female cats (average BW 4.0 kg; age 4 to 6 year) were
181 fed the same commercial dry diet for adult cats (COOP Italia, Bologna, Italy), for 4
182 week before collection of fresh faecal samples. The diet contained the following
183 ingredients: cereals, meat and meat by-products, vegetable by-products, fish by-
184 products, protein plant extract, oils and fat, minerals, and vegetables. The
185 macronutrient composition of the diet (as fed) was the following: water 63 g/kg, crude
186 protein 277 g/kg, ether extract 94 g/kg, crude ash 98 g/kg, starch 397 g/kg, crude fibre
187 33 g/kg, NDF 258 g/kg, ADF 50 g/kg, and ADL 16 g/kg. The same dry food that was
188 fed to the cats used as faecal donors was digested in triplicate (Biagi et al. 2016). After
189 in vitro digestion of the diet fed to the cats, the undigested fraction was dried at 70 °C
190 (23.6 g of undigested residue were obtained from 100 g of food DM) and its chemical
191 composition was the following: crude protein 135 g/kg, ether extract 23 g/kg, starch
192 56 g/kg, crude ash 281 g/kg, NDF 322 g/kg, ADF 133 g/kg, and ADL 53 g/kg.
193 The method used in this experiment reflects the one adopted for the canine experiment.
194

195 ***2.3 Chemical analyses***

196 Analyses of commercial dry food and digested diets were performed according to
197 AOAC International standard methods (AOAC, 2000; method 950.46 for water,
198 method 954.01 for crude protein, method 920.39 for ether extract, method 920.40 for
199 starch, method 942.05 for crude ash, and method 962.09 for crude fibre). Fibre
200 fractions were determined according to the procedure described by Van Soest et al.
201 (1991), where NDF was assayed with a heat stable amylase and expressed inclusive of
202 residual ash, ADF was expressed inclusive of residual ash, and lignin was determined
203 by solubilization of cellulose with sulfuric acid. Ammonia was measured using a
204 commercial kit (Urea/BUN – Color; BioSystems S.A., Spain). Volatile fatty acids

205 were analysed by HPLC. For the determination of biogenic amines, samples were
206 diluted 1:5 with perchloric acid (0.3 M); biogenic amines were later separated by
207 HPLC and quantified through fluorimetry.
208
209 ***2.4 Microbial analysis***
210 A 1-ml portion of fermentation fluid was collected from each vessel and centrifuged
211 at 4 °C for 5 min at 18000 *g*. The supernatant was removed and immediately frozen at
212 – 80°C for further analysis. Bacterial genomic DNA was extracted from remaining
213 pellet using the QIAamp Fast DNA Stool Mini-Kit (QIAGEN GmbH, Germany).
214 Isolated DNA concentration (ng/µl) and purity were measured using a NanoDrop 1000
215 spectrophotometer (Thermo Scientific, Wilmington, DE). Template DNA was diluted
216 to 50 ng/µl and stored at – 20 °C until further analysis. *Escherichia coli* (Malinen
217 2003), *Bifidobacterium* genus (Matsuki et al. 2002), *Lactobacillus genus* (Collier et al.
218 2003), and *Enterococcus genus* (Rinttilä et al. 2004) were quantified via qPCR using
219 specific primers.
220 The qPCR assay was performed using a CFX96 Touch thermal cycler (Bio-
221 Rad, USA). Amplification was performed in duplicate for each bacterial group within
222 each sample. Briefly, the PCR reaction contained 7.5 µl 2X SensiFAST No-ROX PCR
223 Master Mix (Bioline GmbH, Germany), 4.8 µL of nuclease-free water, 0.6 µl of each
224 10 pmol primer, and 1.5 µl of template DNA for a final reaction volume of 15 µl. The
225 amplification cycle was as follows: initial denaturation at 95 °C for 2 min, 95 °C for 5
226 s, primer annealing at 55–61 °C for 10 s and 72 °C for 8 s. The cycle was repeated 40
227 times. A negative control (without the DNA template) was also run for each primer
228 pair. Standard curves were constructed from eight 10-fold dilutions for *Escherichia*
229 *coli, Bifidobacterium* genus, *Lactobacillus genus*, and *Enterococcus genus*. Cycle
230 threshold (Ct) values were plotted against standard curves for quantification of the

231 target bacterial DNA from faecal inoculum. Melting curves were checked after
232 amplification to ensure single product amplification of consistent melting temperature.
233
234 ***2.5 Determination of total volatile compounds (VOCs)***
235 A 1-ml portion of fermentation fluid was collected from each vessel, placed in a 7-ml
236 vial with PTFE/red rubber septa (Supelco, Bellefonte, PA, USA) and capped. Volatile
237 compounds were extracted by headspace-solid phase microextraction (HS-SPME)
238 using a fused-silica fibre (10 mm length) coated with a 50/30 mm thickness of
239 divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) and determined
240 by GC/MS (QP-2010 Plus, Shimadzu, Japan), interfaced with a computerized system
241 for data acquisition (Software GC-MS Solution V. 2.5, Shimadzu, Japan). A RTX-
242 WAX column (30 m, 0.25 mm i.d., 0.25 μm film thickness, Restek, USA) coated with
243 a stationary phase of polyethylene glycol, was used. The SPME fibre was first
244 conditioned by heating it in a GC injection port at 270 °C for 60 min; then, it was
245 inserted into the sample vial through the septum and exposed to the headspace (10 min
246 at 60 °C) of the previously conditioned sample in a water bath at 60 °C for 60 min.
247 Thereafter, the fibre was withdrawn into the needle and transferred to the injection port
248 of the GC/MS system. The SPME fibre was desorbed and maintained in the injection
249 port at 240 °C for 10 min. The sample was injected in split mode at a 1:30 split ratio.
250 Helium was used as the carrier gas at a constant flow rate of 1 mL/min and a linear
251 velocity of 36.2 cm/s. The oven temperature was kept at 40 °C for 10 min, then raised
252 to 200 °C at 3 °C/min, and finally increased to 230 °C at 10 °C/min; final temperature
253 was held for 5 min. The total run time was 74.33 min. Both injector and interface
254 temperatures were set at 240 °C. The ion source temperature was set at 200 °C. The
255 filament emission current was 70 eV. A mass range from 40 to 250 *m/z* was scanned
256 at a rate of 0.25 scans/s. The acquisition and integration modes were Full Scan (TIC)

257 and Single Ion Monitoring (SIM), respectively. Compounds were identified by
258 comparing their mass spectra with those contained in the NIST08 (National Institute
259 of Standards and Technology, Gaithersburg) library and those reported in a previous
260 study (Francioso et al. 2010). In order to control and prevent eventual environmental
261 contamination, a blank injection of fibre and vials was carried out daily.

262

263 ***2.6 Statistical analyses***

264 Data were analysed by 2-way ANOVA, with group YSE and group CT as the main
265 effects; the Newman-Keuls test was used as the post hoc test. Five bottles (n = 5) were
266 set up per treatment; each bottle represented an independent replicate. Differences
267 were considered statistically significant at $p < 0.05$. The principal components analysis
268 (PCA) was used to better understand the data variability. Statistical analyses were
269 performed using Statistica 10.0 software (Stat Soft Italia, Italy).

270

271 **3. Results**

272 ***3.1 Chemical and microbial analyses***

273 *3.1.1 Canine experiment*

274 pH values and concentration of ammonia and biogenic amines after 6 and 24 h of
275 incubation with canine faecal inoculum are shown in Table 1. The pH values at 6 h
276 were unaffected by treatments ($p > 0.05$), while, after 24 h of incubation, pH was
277 significantly higher ($p < 0.001$) in vessels containing group CT. After 6 h of
278 fermentation, ammonia was lowered ($p < 0.05$) by group CT. At 6 h, in the group
279 CT+YSE vessels, the concentration of cadaverine was reduced ($p < 0.001$). After 24 h
280 of incubation, group CT tended to lower the concentration of putrescine ($p = 0.088$)
281 and decreased spermidine ($p = 0.010$). Finally, after 24 h of incubation, group YSE
282 tended to reduce spermine concentrations ($p = 0.066$).

283 Volatile fatty acids concentrations at 6 and 24 h of fermentation with canine
284 faecal inoculum are shown in Table 2. Only traces of n-valeric acid were detected (data
285 not shown). Production of total VFA was decreased ($p < 0.004$) by group CT after 6
286 h, but not after 24 h of incubation. After 6 h of incubation, addition of group CT to the
287 inocula resulted ($p < 0.05$) in lower concentrations of acetate, propionate and
288 isobutyrate; the latter was decreased ($p = 0.009$) also by group YSE. After 24 h of
289 incubation, group CT tended to reduce ($p = 0.083$) the concentrations of propionic
290 acid.

291 The abundances of some bacterial populations in canine faecal inoculum are
292 presented in Table 3. After 6 h, group YSE resulted in an increase ($p = 0.035$) of the
293 presence of *Enterococcus* spp., whereas *E. coli* abundances were lowered by group CT
294 ($p = 0.009$). After 24 h of incubation, the abundances of the assessed microbial
295 populations were not affected by treatments ($p > 0.05$), but group YSE tended to reduce
296 the presence of lactobacilli ($p = 0.053$). Bifidobacteria were inconsistently detected in
297 canine faecal inoculum (data not shown). (Tables 1, 2 and 3 near here).
298
299 *3.1.2 Feline experiment*
300 The values of pH and concentration of ammonia and biogenic amines determined after
301 6 and 24 h of incubation with feline faecal inoculum, are shown in Table 4.

302 After 6 h of incubation, the pH level was reduced ($p = 0.021$) by group YSE,
303 while, at 24 h, pH was higher ($p < 0.001$) in vessels containing group CT. Ammonia
304 concentrations at 6 and 24 h were not affected by treatment ($p > 0.05$). Regarding the
305 concentration of biogenic amines, cadaverine was lowered by group YSE at both 6 (p
306 $= 0.017$) and 24 h (- 20%; $p < 0.001$), while an increase ($p = 0.040$) in spermine
307 concentration was observed at 24 h in the group YSE vessels.

308 Concentrations of volatile fatty acids at 6 and 24 h of fermentation with feline
309 faecal inoculum are shown in Table 5. After 6 h, concentration of total VFA tended to
310 be lower for both group CT ($p = 0.092$) and group YSE ($p = 0.052$) treatments, while,
311 at 24 h, total VFA concentration was significantly reduced ($p = 0.002$) by group CT.
312 At 6 h, the presence of group YSE resulted in lower concentrations of acetic ($p = 0.010$)
313 and valeric acids ($p = 0.006$). In bottles containing group CT, a reduction ($p = 0.010$)
314 of the concentration of propionic acid was observed at 6 h, while a decrement of acetate
315 ($p = 0.044$), propionate ($p < 0.0001$) and isovalerate ($p = 0.039$) was noted at 24 h.
316 Similarly, at 24 h, group CT tended to reduce ($p = 0.095$) the concentration of butyrate.
317 Abundances of investigated microbial populations in the feline faecal inoculum
318 are shown in Table 6. At 6 h, the presence of enterococci was reduced by group
319 CT+YSE ($p = 0.008$). (Tables 4, 5 and 6 near here).
320

321 ***3.2 Volatile compounds (VOCs) detected by HS-SPME-GC/MS analysis***

322 In all samples, a total of 130 different metabolites were identified, of which 36 were
323 cat-related and 20 were dog-related (Supplemental Table 1). Most of VOCs that have
324 been identified belong to the families of esters and alcohols; however, after 6 h of
325 incubation, one of the most represented metabolites in feline and canine faecal
326 inoculum was indole, which accounted for 47.1% and 39.7% of total VOCs area,
327 respectively. After 24 h of fermentation, indole proportions decreased in both dog and
328 cat inoculum (22.2% and 17.8% of total VOCs area, respectively). Other
329 representative metabolites detected in both dog and cat vessels after 6 h of incubation,
330 were ethanol, 1-propanol, 1-butanol, 3-methyl-1-butanol, and 1-hexanol (belonging to
331 the family of alcohols), while phenol and dimethyl trisulphide were more predominant
332 in dog faecal inoculum. After 24 h of fermentation, phenol was the most abundant
333 metabolite detected in the dog faecal inoculum (32.9% of total VOCs area), followed

334 by indole, dimethyl trisulphide and dimethyl disulphide (22.2%, 11.2% and 6.7% of
335 total VOCs area, respectively).
336
337 *3.2.1 Canine experiment*
338 Absolute area of VOCs obtained from the HS-SPME-GC/MS analysis of dog faecal
339 inoculum is shown in Supplemental Table 2. After 6 h of incubation, hydrogen
340 sulphide was higher ($p < 0.05$) in group CT vessels. Regarding aldehyde compounds,
341 the addition of group CT resulted in higher amounts of caproaldehyde ($p = 0.024$) and
342 nonaldehyde ($p = 0.017$) after 6 h of fermentation, and higher amounts of
343 isovaleraldehyde at both 6 (p < 0.001) and 24 h (p < 0.001). Similarly,
344 isovaleraldehyde amount was increased ($p < 0.05$) by group YSE at 24 h. On the
345 contrary, after 6 h of incubation, a significant decrease ($p = 0.025$) of caproaldehyde
346 was observed in YSE vessels. Quinoline was decreased by the presence of group CT
347 at both 6 and 24 h ($p < 0.05$), while phenol was increased at 6 h by group CT ($p =$
348 0.007).
349 After 24 h of incubation, a decrease of the amount of dimethyl sulphide ($p =$
350 0.058) and dimethyl disulphide ($p < 0.05$) was observed in group CT batches;
351 similarly, group CT decreased the amount of acetic acid butyl ester ($p < 0.001$), acetic
352 acid propyl ester ($p = 0.003$) and total esters ($p = 0.006$).
353
354 *3.2.2 Feline experiment*
355 Absolute areas of the significantly different VOCs detected by HS-SPME-GC/MS
356 analysis of feline faecal inoculum are shown in Supplemental Table 3. After 6 h of
357 incubation, lower amounts of indole ($p < 0.001$), 1-butanol ($p = 0.008$), 1-butanol, 3-
358 methyl ($p < 0.01$), 1-pentanol ($p = 0.019$) and total alcohols ($p = 0.035$), were observed
359 in vessels containing group CT. Conversely, group CT increased ($p < 0.05$) the amount

360 of hydrogen sulphide after 6 h of incubation. Addition of YSE resulted in a decreasing
361 trend in trimethylamine ($p = 0.069$) and ethanol ($p = 0.073$) amounts.

362 After 24 h, hydrogen sulphide amount was lower ($p < 0.05$) in batches where
363 the combination CT+YSE had been added, while a reduction tendency ($p = 0.092$) of
364 this metabolite was observed in group YSE vessels. Likewise, the addition of CT+YSE
365 to the inocula tended to reduce total aldehydes ($p = 0.095$), 2-propanone ($p = 0.071$)
366 and acetic acid ethyl ester ($p = 0.095$). After 24 h of incubation, a decrease ($p < 0.05$)
367 of propanethioate-S-methyl and acetic acid 3-methylbutyl ester was observed in group
368 CT vessels. On the contrary, the amount of *p*-cresol was increased significantly ($p =$
369 0.002) by group CT.

370

371 ***3.3 Principal Component Analysis (PCA)***

372 *3.3.1 PCA of canine experiment*

373 To evaluate the effect of CT and YSE addition on VOCs formation and to define which
374 compounds were the most representative of variance, the data were subjected to PCA.
375 When samples of dog faecal inoculum were tested, the first two principal components
376 reached 77.08% of variance (Supplemental Figure 1). The factor 1 explains 65.63% of
377 variance; in particular, it is possible to observe that sulphur compounds, as well as
378 acetic acid esters, alcohols, organic acids and quinolone, are inversely correlated to
379 indole and carbon disulphide. In addition, a third cluster consisting mainly of
380 aldehydes (isovaleraldehyde, caproaldehyde, nonanal, benzaldehyde and 2-
381 methylbutyraldehyde) was more correlated to Factor 2 (11.45% of variance) (Figure
382 2). Considering the Biplot (Supplemental Figure 2), results at 6-h were well separated
383 from the 24-h ones; in particular, the 6-h group CT assay was completely segregated
384 from the other treatments, while all 24-h assays were plotted in the same cluster and

385 were mainly characterized by the presence of sulphur compounds, alcohols and acetic
386 acid esters.
387 In order to better understand the variance of results, the PCA of all determined
388 parameters from the canine experiment was carried out. As reported in Biplot (Figure
389 1), a total of 86.26% of variance was comprised in the first two principal components,
390 of which PC1 explains 69.98% of total variance. In addition, it is possible to observe
391 the presence of several clusters; in fact, PC2 evidences how lactobacilli, enterococci,
392 total organic acids and 2-propanone were inversely correlated to the main aldehydes
393 (e.g. caproaldehyde, benzaldehyde, etc.) and the 6-h control sample. On the other hand,
394 the 24-h samples did not separate and were mainly characterized by the VOCs
395 presence as reported above, which thus demonstrate a non-significant effect of group
396 CT and group YSE after 24 h. (Figure 1 near here).
397
398 *3.3.2 PCA of feline experiment*
399 A PCA analysis of feline faecal VOCs was also carried out. Supplemental Figure 3
400 reports the component loading in the cat experiment; the first two principal
401 components explained 81.78% of the total variance. In general, aldehydes, alcohols,
402 organic acids and sulphur compounds were more correlated to Factor 1, which
403 explained 67.26% of variance, whereas acetic acid esters, phenol and indole were
404 correlated to Factor 2, which explained 14.52% of variance. In Supplemental Figure
405 4, a clear separation between 6-h and 24-h results is evidenced. After 6 h, group YSE
406 and group CT treatments were mainly characterized by the presence of aldehydes (3-
407 methylbenzaldehyde and isovaleraldehyde) and alcohols (3-methyl-1-butanol and
408 ethanol), while VOCs at 24 h are mainly depicted by acetic acid esters, phenol, sulphur
409 compounds, butyraldehyde and benzaldehyde. However, it might be pointed out that,

410 at 6 h, the combination YSE+CT was inversely correlated to the concentrations of
411 indole, acetic acid propyl- and ethyl-esters and methyl propane thioate.
412 In addition, the PCA of all determined parameters was carried out (Figure 2).
413 The 73.54% of total variance explained by the first two principal components, of which
414 PC2 was more correlated to *Escherichia coli*, ethyl acetate and ketones. As shown in
415 Figure 2, different clusters could be distinguished, in particular the 6-h samples were
416 all gathered in the same cluster, characterized by aldehydes and ethanol, and inversely
417 correlated to the presence of lactobacilli and organic acids. On the other hand, the 24-
418 h samples (except for CTRL 24h) were mainly depicted by the presence of ammonia,
419 short-chain alcohols and sulphur compounds. (Figure 2 near here).
420
421 **4. Discussion**
422 The results from the present *in vitro* study showed that *Y. schidigera* extract and
423 chestnut tannins were able to exert some influence on the metabolism of canine and
424 feline faecal microbiota.
425
426 ***4.1 pH values, SCFA production and bacterial protein catabolism***
427 It is known that a low intestinal pH helps to improve the health of the gut mucosa by
428 reducing unwanted bacteria and absorption of ammonia, while higher intestinal pH is
429 thought to promote the growth of detrimental microorganisms (Zentek et al. 2013).
430 When incubated with both canine and feline faecal inocula, tannins increased the pH
431 of the fermentation fluid at 24 h, presumably as a consequence of the reduction of total
432 VFA production. In fact, addition of CT to canine and feline slurries resulted in lower
433 concentrations of acetate and propionate. Bravo et al. (1994) similarly reported that
434 fermentation of tannic acid (1 g/l) with a rat caecal inoculum led to a decrease in acetic
435 and propionic acid concentrations, while tannic acid at 2.5 g/l resulted in the total

436 suppression of acetic acid production, suggesting that tannic acid could be a specific
437 inhibitor of acetate-producing bacteria. A decrease of SCFA concentrations in the
438 colonic lumen may lead to mucosal atrophy as a consequence of a lack of energy for
439 colonocytes (Machiels et al. 2014).

440 Compared with CTRL, group CT and group YSE significantly reduced the
441 concentration of isobutyric acid in canine inocula, while group CT lowered the amount
442 of isovalerate in feline inocula after 6 h. Isobutyric and isovaleric acid, as well as 2-
443 methylbutyrate, mainly derive from the degradation of valine, isoleucine and leucine
444 (Hughes et al. 2000), and are used as faecal markers for bacterial protein catabolism.
445 In the first experiment, ammonia concentration, as well as spermidine concentration,
446 were reduced by group CT, while the association CT+YSE decreased the amount of
447 cadaverine. In presence of feline faecal inocula, group CT failed to decrease the
448 amount of biogenic amines, even though group YSE reduced cadaverine but increased
449 spermine concentration. Ammonia and biogenic amines are also products which derive
450 from proteolytic reactions; in particular, biogenic amines are generally formed through
451 the decarboxylation of specific free amino acids by microbial decarboxylases (Ku et
452 al. 2013). The inhibitory effects of tannins on microbial proteolysis had already been
453 observed by Biagi et al. (2010) in piglets (both *in vitro* and *in vivo*), and by Hassanat
454 and Benchaar (2013) in presence of ruminal inoculum. *Yucca schidigera* is commonly
455 used as a dietary additive in livestock and companion animals (Lowe et al. 1997; Lowe
456 and Kershaw 1997) for the reduction of ammonia and faecal odour in animal excreta.
457 Several mechanisms have been proposed to explain the effect of *Y. schidigera* extracts
458 on ammonia concentrations in animal faeces, among which are the ability to inhibit
459 the enzyme urease (Balog et al. 1994), to bind directly ammonia (McCrory and Hobbs
460 2001) and to inhibit specific bacterial strains (Patra and Saxena, 2009). However, in
461 the present study, YSE failed to reduce ammonia concentrations. Similarly, ammonia

462 content of caecal material did not decrease in rats receiving sarsaponin (steroidal
463 glycosides extracted from *Y. schidigera*) with the diet (120 mg/kg of diet; Preston et
464 al., 1987).
465
466 ***4.2 Faecal microbiota***
467 In this study, the addition of YSE to canine faecal inoculum resulted in an increase of
468 enterococci but the same effect was not observed when group YSE was incubated in
469 presence of the feline faecal inoculum. There is no evidence in the literature suggesting
470 that *Y. schidigera* may act as a prebiotic so that, at present, we do not know if the
471 increment of enterococci was the consequence of a direct effect of YSE on their growth
472 or the result of the inhibition of enterococci antagonists by the saponins contained in
473 the YSE (Killeen et al. 1998). Among the tested substrates, group CT resulted in a
474 reduction of *E. coli* in the first experiment, while, in presence of feline faecal inoculum,
475 *E. coli* abundances tended to be lower when CT was used in association with YSE.
476 These results are not in agreement with those of Biagi et al. (2010), who reported a
477 lack of antibacterial activity of chestnut tannins against coliforms, both *in vitro* and *in*
478 *vivo*. It has been observed that yucca saponins may exhibit antibacterial effects on *E.*
479 *coli* (Sen et al. 1998), but other authors did not report any bacteriostatic effect of *Y.*
480 *schidigera* on pure cultures of *E. coli* (Killeen et al. 1998). However, Wang et al.
481 (2000) showed that saponins from different sources, including *Y. schidigera*, are more
482 effective against gram-positive than gram-negative bacteria (e.g. *E. coli*). The
483 reduction of the presence of *E. coli* in the animals' intestine may be considered
484 beneficial. However, when chestnut tannins were added to canine faecal inocula, minor
485 effects were observed only after 6 h of incubation, which are probably without any
486 physiological importance.
487

488 ***4.4 Volatile organic compounds in faecal inocula***

489 To the best of our knowledge, our results provide the first characterization of VOCs
490 profile of canine and feline faecal inocula by HS-SPME-GC/MS. A great variety of
491 VOCs was found in the fermentation fluids. The addition of chestnut tannins to the
492 canine faecal inocula led to an increase of hydrogen sulphide and a decrease of
493 dimethyl sulphide and dimethyl disulphide, making the interpretation of these results
494 very difficult, as both metabolites are produced from the catabolism of S-containing
495 amino acids. Hydrogen sulphide is the main product of sulphate-reducing bacterial
496 catabolism of cysteine and, when present at high concentrations, may inhibit the
497 oxidation of butyrate by the colonic epithelium (Roediger et al. 1993). In general,
498 hydrogen sulphide is methylated by the colonic epithelium to less harmful products,
499 such as methanethiol and dimethyl sulphide (Weiseger et al. 1980). In the feline
500 experiment, an increase of carbon sulphide and hydrogen sulphide was observed at 6
501 h in the vessels containing CT, while hydrogen sulphide was decreased at 24 h by
502 group CT+YSE. In a previous *in vitro* study with dog faecal inoculum, the addition of
503 *Y. schidigera* (0.17 g/l) resulted in lower (- 38%) hydrogen sulphide but did not affect
504 total gas production (Giffard et al. 2001). In the study by Swanson et al. (2002), feeding
505 dogs with a *Lactobacillus acidophilus* supplemented diet tended to increase dimethyl
506 sulphide, methanethiol and hydrogen sulphide faecal concentrations. The ability of
507 some species of *Lactobacillus* spp. to produce hydrogen sulphide has been reported by
508 Arici et al. (2004). In the already cited study by Swanson et al. (2002), dogs fed fructo-
509 oligosaccharides (FOS) at 2 g/d had the lowest faecal concentrations of S-compounds.
510 Conversely, in the study by Hesta et al. (2005), the administration of FOS at 31 g/kg
511 of diet (on DM basis) failed to reduce the faecal concentration of sulphur compounds
512 in cats receiving a protein-restricted diet. Although in this study S-compounds
513 accounted only for a small part of identified chemicals (1.40% and 2.65% of total

514 VOCs area after 6 h of incubation in presence of feline and canine faecal inocula,
515 respectively), they are in general considered responsible for the distinctive bad odour
516 of faeces. In fact, as reported by Hesta et al. (2005), S-containing components have an
517 important impact on faecal odour, since six out of 10 compounds with the lowest odour
518 detection threshold (degree of sensitivity of humans) contain sulphur.

519 While the utilization of CT led to contradictory results with regard to the
520 concentrations of S-compounds, the experimental substrates used in this study were
521 more effective in limiting the presence of N-compounds. In fact, group CT lowered
522 the amount of quinoline (in presence of canine faecal inoculum) and indole (when
523 incubated with feline faecal inoculum), while group YSE tended to lower the amount
524 of trimethylamine in presence of cat faecal inoculum. Quinoline and indole are
525 aromatic heterocyclic organic compounds; indole derives from microbial degradation
526 of tryptophan in the colon (Shimada et al. 2013). In the past, indole was suspected to
527 contribute to colon carcinogenesis, but Shimada et al. (2013) recently attributed to
528 indole immunomodulatory and anti-inflammatory properties on intestinal epithelial
529 cells. However, in a study by Nowak and Libudzisz (2006), indole showed negative
530 effects on the *in vitro* viability of lactic acid bacteria. Trimethylamine is a tertiary
531 amine derived from the bacterial catabolism of choline and, to a less extent, carnitine
532 (Russell et al. 2013); trimethylamine can be further metabolized by gut microbiota to
533 trimethylamine-N-oxide, a catabolite recently considered to be involved in human
534 colorectal cancer pathogenesis (Xu et al. 2015). In the present study, trimethylamine
535 levels tended to be reduced by group YSE.

536 The utilization of CT resulted in higher concentrations of phenol and *p*-cresol
537 (4-methylphenol) in canine and feline faecal inocula, respectively. Phenol and *p*-cresol
538 are recognized as potentially harmful by-products deriving from the bacterial
539 catabolism of aromatic amino acids and are correlated with the development of cancer

540 in the human colon (Nowak and Libudzisz 2006). It has been shown that numerous
541 mammals host bacteria that are able to degrade hydrolyzable tannins (Kohl et al. 2015).
542 Hydrolyzable tannins are polyesters consisting of a carbohydrate core and phenolic
543 acids (mainly gallic and ellagic acids), which are characterized by a phenolic ring and
544 a carboxyl group. Based on our results, it may be supposed that the phenolic acids
545 released from the tannins may be further metabolized to phenol and *p*-cresol by the gut
546 microbiota. The slightly higher amount of phenol detected in vessels containing YSE
547 and canine faecal inoculum may be the consequence of microbial degradation of
548 polyphenolic compounds (resveratrol and yuccaols) that can be found in *Y. schidigera*.
549 However, the microbial metabolism of resveratrol in the large intestine is still
550 uncertain (Selma et al. 2009). Interestingly, the association CT+YSE resulted in a
551 strong reduction of phenol concentrations, suggesting a synergistic effect of CT and
552 YSE for which we do not have an explanation.

553 The observed reduction of the amounts of ketones and alcohols as a result of
554 CT addition, seems to suggest a decreased microbial activity induced by tannins. In
555 fact, ketones can derive from the microbial catabolism of fatty acids, while alcohols
556 can be produced during sugar fermentation or reduction of organic acids by bacteria
557 (de Lacy Costello et al. 2014).

558 Esters, the most numerous chemical class (36 identified compounds) in our
559 samples, derive from the esterification of alcohols and fatty acids by the gut microbiota
560 or by the esterase activity of the enterocytes (Garner et al. 2007). The lowering effect
561 of group CT on esters abundance in the canine and, to a lesser extent, feline faecal
562 inocula may be related, as observed by Daniel et al. (1991) in rats, to the inhibition of
563 esterase activity by tannins.

564 Changes in the composition and abundances of VOCs in the headspace of
565 vessels that were observed in this study can be directly related to the gut microbiota

metabolism and to the presence of the experimental substrates. Feeding *Y. schidigera* to dogs and cats has been demonstrated to reduce faecal malodour by sensory testing (Lowe and Kershaw 1997), while a concurrent study with dogs and cats showed changes in the faecal concentrations of several volatile components after *Y. schidigera* extract supplementation (Lowe et al. 1997). However, the authors did not find a direct correlation between the changes in the amounts of VOCs and the aroma amelioration. In fact, faecal odour is the resultant of absolute and relative concentrations of several compounds, the possible interactions among different chemicals and the impact of both texture and microstructure of faeces on release of odour compounds (Hesta et al. 2005; de Lacy Costello et al. 2014).

5. Conclusions

The results from the present study show that *Y. schidigera* extract and tannins from chestnut wood exert different effects on the composition and metabolism of canine and feline faecal microbiota. Although a decrease in malodorous compounds was observed, it is not possible to state whether or not the tested substrates lowered the inoculum odour. However, the dietary supplementation of *Y. schidigera* and tannins to dogs and cats may be beneficial due to the reduction of some potentially toxic volatile metabolites.

Acknowledgments

Dr. Anna Miserocchi is acknowledged for technical assistance.

Disclosure statement

No potential conflict of interest was reported by the authors.

592 **Funding**

593 This research did not receive any specific grant from funding agencies in the public,
594 commercial, or not-for-profit sectors.

595

596 **References**

597 [AOAC] Association of Official Analytical Chemists. 2000. Official methods of
598 analysis, 17th ed. AOAC, Gaithersburg (MD).
599 Arici M, Bilgin B, Sagdic O, Ozdemir C. 2004. Some characteristics of Lactobacillus
600 isolates from infant faeces. Food Microbiol. 21:19–24.
601 Aura AM. 2008. Microbial metabolism of dietary phenolic compounds in the colon.
602 Phytochem Rev. 7:407–429.
603 Balog JM, Anthony NB, Wall CW, Walker RD, Rath NC, Huff WE. 1994. Effect of
604 a urease inhibitor and ceiling fans on ascites in broilers.: 2. blood variables, ascites
605 scores, and body and organ weights. Poult Sci. 73:810–816.
606 Biagi G, Cipollini I, Grandi M, Pinna C, Vecchiato CG, Zaghini G. 2016. A new in
607 vitro method to evaluate digestibility of commercial diets for dogs. Ital J Anim
608 Sci.:1–9.
609 Biagi G, Cipollini I, Paulicks BR, Roth FX. 2010. Effect of tannins on growth
610 performance and intestinal ecosystem in weaned piglets. Arch Anim Nutr. 64:121–
611 135.
612 Bravo L, Abia R, Eastwood MA, Saura-Calixtol F. 1994. Degradation of
613 polyphenols (catechin and tannic acid) in the rat intestinal tract. Effect on colonic
614 fermentation and faecal output. Br J Nutr. 71:933–946.
615 Collier CT, Smiricky-Tjardes MR, Albin DM, Wubben JE, Gabert VM, Deplancke
616 B, Bane D, Anderson DB, Gaskins HR. 2003. Molecular ecological analysis of
617 porcine ileal microbiota responses to antimicrobial growth promoters. J Anim Sci.

618 81:3035–3045.
619 Daniel EM, Ratnayake S, Kinstle T, Stoner GD. 1991. The effects of pH and rat
620 intestinal contents on the liberation of ellagic acid from purified and crude
621 ellagitannins. J Nat Prod. 54:946–952.
622 Duffy CF, Killeen GF, Connolly CD, Power RF. 2001. Effects of dietary
623 supplementation with Yucca schidigera Roezl ex Ortgies and its saponin and non-
624 saponin fractions on rat metabolism. J Agric Food Chem. 49:3408–3413.
625 Francioso O, Rodriguez-Estrada MT, Montecchio D, Salomoni C, Caputo A,
626 Palenzona D. 2010. Chemical characterization of municipal wastewater sludges
627 produced by two-phase anaerobic digestion for biogas production. J Hazard Mater.
628 175:740–746.
629 Garner CE, Smith S, de Lacy Costello B, White P, Spencer R, Probert CSJ, Ratcliffe
630 NM. 2007. Volatile organic compounds from feces and their potential for diagnosis
631 of gastrointestinal disease. FASEB J. 21:1675–1688.
632 Giffard CJ, Collins SB, Stoodley NC, Butterwick RF, Batt RM. 2001.
633 Administration of charcoal, Yucca schidigera, and zinc acetate to reduce malodorous
634 flatulence in dogs. J Am Vet Med Assoc. 218:892–896.
635 Hassanat F, Benchaar C. 2013. Assessment of the effect of condensed (acacia and
636 quebracho) and hydrolysable (chestnut and valonea) tannins on rumen fermentation
637 and methane production in vitro. J Sci Food Agric. 93:332–339.
638 Hesta M, Hoornaert E, Verlinden A, Janssens GPJ. 2005. The effect of oligofructose
639 on urea metabolism and faecal odour components in cats. J Anim Physiol Anim Nutr
640 (Berl). 89:208–214.
641 Hughes R, Magee EA, Bingham S. 2000. Protein degradation in the large intestine:
642 relevance to colorectal cancer. Curr Issues Intest Microbiol. 1:51–58.
643 Jamroz D, Wiliczkiewicz A, Skorupińska J, Orda J, Kuryszko J, Tschirch H. 2009.

644 Effect of sweet chestnut tannin (SCT) on the performance, microbial status of
645 intestine and histological characteristics of intestine wall in chickens. Br Poult Sci.
646 50:687–699.
647 Killeen GF, Madigan CA, Connolly CR, Walsh GA, Clark C, Hynes MJ, Timmins
648 BF, James P, Headon DR, Power RF. 1998. Antimicrobial saponins of Yucca
649 schidigera and the implications of their in vitro properties for their in vivo impact. J
650 Agric Food Chem. 46:3178–3186.
651 Kohl KD, Stengel A, Dearing MD. 2015. Inoculation of tannin-degrading bacteria
652 into novel hosts increases performance on tannin-rich diets. Environ Microbiol.
653 18:1720–1729.
654 Ku BS, Mamuad LL, Kim SH, Jeong CD, Soriano AP, Lee HI, Nam KC, Ha JK, Lee
655 SS. 2013. Effect of γ-Aminobutyric Acid (GABA) producing bacteria on in vitro
656 rumen fermentation, biogenic amine production and anti-oxidation using corn meal
657 as substrate. Asian-Australasian J Anim Sci. 26:804–811.
658 de Lacy Costello B, Amann A, Al-Kateb H, Flynn C, Filipiak W, Khalid T, Osborne
659 D, Ratcliffe NM. 2014. A review of the volatiles from the healthy human body. J
660 Breath Res. 8:14001.
661 Lowe J., Kershaw S. 1997. The ameliorating effect of Yucca schidigera extract on
662 canine and feline faecal aroma. Res Vet Sci. 63:61–66.
663 Lowe J., Kershaw S., Taylor A., Linforth RS. 1997. The effect of Yucca schidigera
664 extract on canine and feline faecal volatiles occurring concurrently with faecal aroma
665 amelioration. Res Vet Sci. 63:67–71.
666 Machiels K, Joossens M, Sabino J, De Preter V, Arijs I, Eeckhaut V, Ballet V, Claes
667 K, Van Immerseel F, Verbeke K, et al. 2014. A decrease of the butyrate-producing
668 species Roseburia hominis and Faecalibacterium prausnitzii defines dysbiosis in
669 patients with ulcerative colitis. Gut. 63:1275–1283.

670 Malinen E. 2003. Comparison of real-time PCR with SYBR Green I or 5'-nuclease
671 assays and dot-blot hybridization with rDNA-targeted oligonucleotide probes in
672 quantification of selected faecal bacteria. Microbiology. 149:269–277.
673 Matsuki T, Watanabe K, Fujimoto J, Miyamoto Y, Takada T, Matsumoto K, Oyaizu
674 H, Tanaka R. 2002. Development of 16S rRNA-gene-targeted group-specific primers
675 for the detection and identification of predominant bacteria in human feces. Appl
676 Environ Microbiol. 68:5445–5451.
677 McAllister T., Annett C., Cockwill C., Olson M., Wang Y, Cheeke P. 2001. Studies
678 on the use of Yucca schidigera to control giardiosis. Vet Parasitol. 97:85–99.
679 McCrory DF, Hobbs PJ. 2001. Additives to reduce ammonia and odor emissions
680 from livestock wastes. J Environ Qual. 30:345–355.
681 Miyakoshi M, Tamura Y, Masuda H, Mizutani K, Tanaka O, Ikeda T, Ohtani K,
682 Kasai R, Yamasaki K. 2000. Antiyeast steroidal saponins from Yucca schidigera
683 (mohave yucca), a new anti-food-deteriorating agent. J Nat Prod. 63:332–338.
684 Nowak A, Libudzisz Z. 2006. Influence of phenol, p-cresol and indole on growth and
685 survival of intestinal lactic acid bacteria. Anaerobe. 12:80–84.
686 Patra AK, Saxena J. 2009. The effect and mode of action of saponins on the
687 microbial populations and fermentation in the rumen and ruminant production. Nutr
688 Res Rev. 22:204–219.
689 Pinna C, Biagi G. 2014. The utilisation of prebiotics and synbiotics in dogs. Ital J
690 Anim Sci. 13:169–178.
691 Preston RL, Bartle SJ, May T, Goodall SR. 1987. Influence of sarsaponin on growth,
692 feed and nitrogen utilization in growing male rats fed diets with added urea or
693 protein. J Anim Sci. 65:481–487.
694 Rinttilä T, Kassinen A, Malinen E, Krogius L, Palva A. 2004. Development of an
695 extensive set of 16S rDNA-targeted primers for quantification of pathogenic and

696 indigenous bacteria in faecal samples by real-time PCR. J Appl Microbiol. 97:1166–
697 1177.
698 Rochus K, Janssens GPJ, Hesta M. 2014. Dietary fibre and the importance of the gut
699 microbiota in feline nutrition: a review. Nutr Res Rev. 27:295–307.
700 Roediger WE, Duncan A, Kapaniris O, Millard S. 1993. Reducing sulfur compounds
701 of the colon impair colonocyte nutrition: implications for ulcerative colitis.
702 Gastroenterology. 104:802–809.
703 Russell WR, Hoyles L, Flint HJ, Dumas ME. 2013. Colonic bacterial metabolites and
704 human health. Curr Opin Microbiol. 16:246–254.
705 Selma MV, Espín JC, Tomás-Barberán FA. 2009. Interaction between phenolics and
706 gut microbiota: role in human health. J Agric Food Chem. 57:6485–6501.
707 Sen S, Makkar HPS, Muetzel S, Becker K. 1998. Effect of Quillaja saponaria
708 saponins and Yucca schidigera plant extract on growth of Escherichia coli. Lett Appl
709 Microbiol. 27:35–38.
710 Shimada Y, Kinoshita M, Harada K, Mizutani M, Masahata K, Kayama H, Takeda
711 K. 2013. Commensal bacteria-dependent indole production enhances epithelial
712 barrier function in the colon. PLoS ONE. 8:e80604.
713 Van Soest PJ, Robertson JB, Lewis BA. 1991. Methods for dietary fiber, neutral
714 detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy
715 Sci. 74:3583–3597.
716 Sunvold GD, Fahey GC, Merchen NR, Reinhart GA. 1995. In vitro fermentation of
717 selected fibrous substrates by dog and cat fecal inoculum: influence of diet
718 composition on substrate organic matter disappearance and short-chain fatty acid
719 production. J Anim Sci. 73:1110–1122.
720 Swanson KS, Grieshop CM, Flickinger EA, Bauer LL, Chow J, Wolf BW, Garleb
721 KA, Fahey GC. 2002. Fructooligosaccharides and Lactobacillus acidophilus modify

722 gut microbial populations, total tract nutrient digestibilities and fecal protein
723 catabolite concentrations in healthy adult dogs. J Nutr. 132:3721–3731.
724 Wang Y, McAllister TA, Yanke LJ, Cheeke PR. 2000. Effect of steroidal saponin
725 from Yucca schidigera extract on ruminal microbes. J Appl Microbiol. 88:887–896.
726 Weiseger R, Pinkus L, Jakoby W. 1980. Thiol-S-methyltransferase: suggested role in
727 detoxification of intestinal hydrogen sulphide. Biochem Pharmacol. 29:2885–2887.
728 Windisch W, Schedle K, Plitzner C, Kroismayr A. 2008. Use of phytogenic products
729 as feed additives for swine and poultry. J Anim Sci. 86:E140-148.
730 Xu R, Wang Q, Li L. 2015. A genome-wide systems analysis reveals strong link
731 between colorectal cancer and trimethylamine N-oxide (TMAO), a gut microbial
732 metabolite of dietary meat and fat. BMC Genomics. 16:S4.
733 Zentek J, Ferrara F, Pieper R, Tedin L, Meyer W, Vahjen W. 2013. Effects of dietary
734 combinations of organic acids and medium chain fatty acids on the gastrointestinal
735 microbial ecology and bacterial metabolites in the digestive tract of weaning piglets.
736 J Anim Sci. 91:3200-3210.
737
738

Table 1. pH values and mean concentrations (n = 5) of ammonia [mmol/l] and biogenic amines [µmol/l] after 6 and 24 h of an in vitro incubation of canine faecal inoculum with a control diet to which different substrates were added.

	Group CTRL	Group CT	Group YSE	Group CT + YSE	Pooled SEM	ANOVA *p*-value		
						Group CT	Group YSE	Group CT + YSE
At 6 h								
pH	6.37	6.37	6.36	6.36	0.01	0.955	0.436	0.822
NH_3	38.5	37.1	38.7	36.8	0.48	0.015	0.979	0.641
Putrescine	928	1030	1036	854	42.3	0.634	0.684	0.104
Cadaverine	221	225	267	140	15.7	< 0.001	0.219	< 0.001
Spermidine	32.2	32.2	29.6	34.6	1.66	0.151	0.953	0.151
Spermine	18.0	17.0	15.6	19.4	1.48	0.357	0.990	0.124
At 24 h								
pH	6.12	6.19	6.09	6.19	0.001	< 0.001	0.186	0.253
NH_3	38.2	40.8	41.3	43.8	2.77	0.366	0.287	0.980
Putrescine	1336	1326	1497	1318	52.1	0.088	0.162	0.123
Cadaverine	322	335	342	312	20.5	0.684	0.950	0.310
Spermidine	40.0	33.6	40.6	30.4	2.84	0.010	0.653	0.513
Spermine	24.6	21.0	20.0	16.2	2.38	0.140	0.066	0.967

Table 2. Mean concentrations (n = 5) of VFA [mmol/l] after 6 and 24 h of an *in vitro* incubation of canine faecal inoculum with a control diet to which different substrates were added.

	Group CTRL	Group CT	Group YSE	Group CT + YSE	Pooled SEM	ANOVA *p*-value		
						Group CT	Group YSE	Group CT + YSE
At 6 h								
Acetic acid	12.0	11.1	11.7	10.7	0.30	0.016	0.373	0.895
Propionic acid	9.86	8.62	9.40	8.95	0.26	0.019	0.837	0.232
Isobutyric acid	1.11	0.67	0.61	0.42	0.10	0.023	0.009	0.310
n-Butyric acid	4.88	4.41	4.70	4.47	0.18	0.138	0.795	0.612
Isovaleric acid	0.90	0.87	0.87	0.66	0.12	0.193	0.199	0.321
Total VFA	28.8	25.7	27.3	25.2	0.60	0.004	0.215	0.528
At 24 h								
Acetic acid	16.3	14.9	16.7	16.1	0.86	0.276	0.389	0.651
Propionic acid	14.1	11.8	15.5	13.8	0.82	0.083	0.172	0.343
Isobutyric acid	1.07	1.02	1.30	1.22	0.13	0.587	0.109	0.927
n-Butyric acid	5.84	5.78	6.09	6.26	0.41	0.893	0.399	0.771
Isovaleric acid	1.77	1.81	1.87	1.86	0.15	0.910	0.636	0.887
Total VFA	39.3	35.4	40.5	39.4	2.24	0.286	0.262	0.553

Table 3. Microbial populations [log 10 DNA copies/ml] detected by qPCR in canine faecal inoculum with a control diet to which different substrates were added. Values are the means of 5 bottles per treatment.

	Group CTRL	Group CT	Group YSE	Group CT + YSE	Pooled SEM	ANOVA *p*-value		
						Group CT	Group YSE	Group CT + YSE
At 6 h								
Lactobacillus spp.	8.06	6.35	7.11	7.06	0.54	0.132	0.837	0.157
Enterococcus spp.	7.38	6.36	9.44	8.22	0.82	0.206	0.035	0.908
E. coli	5.19	4.74	5.95	4.68	0.28	0.009	0.246	0.175
At 24 h								
Lactobacillus spp.	8.41	7.74	7.31	7.54	0.30	0.500	0.053	0.170
Enterococcus spp.	8.10	8.77	7.12	7.86	1.01	0.509	0.381	0.974
E. coli	5.33	4.93	4.84	5.37	0.33	0.857	0.942	0.193

Table 4. pH values and mean concentrations (n = 5) of ammonia [mmol/l] and biogenic amines [µmol/l] after 6 and 24 h of an in vitro incubation of feline faecal inoculum with a control diet to which different substrates were added.

	Group CTRL	Group CT	Group YSE	Group CT + YSE	Pooled SEM	ANOVA p-value		
						Group CT	Group YSE	Group CT + YSE
At 6 h								
pH	6.28	6.28	6.24	6.26	0.01	0.377	0.021	0.372
NH_3	33.4	34.9	34.0	33.5	0.38	0.571	0.664	0.233
Putrescine	187	200	190	179	7.74	0.860	0.188	0.074
Cadaverine	243	245	181	196	21.3	0.689	0.017	0.774
Spermidine	29.0	30.8	32.1	30.4	1.99	0.795	0.641	0.503
Spermine	5.60	5.50	5.00	4.04	0.52	0.355	0.083	0.451
At 24 h								
pH	6.10	6.14	6.05	6.17	0.01	< 0.0001	0.344	0.004
NH_3	52.2	50.9	48.7	52.0	1.68	0.777	0.744	0.536
Putrescine	303	379	373	304	23.8	0.872	0.924	0.007
Cadaverine	265	235	211	212	9.77	0.142	<0.001	0.132
Spermidine	32.0	28.4	27.4	34.4	2.68	0.534	0.797	0.065
Spermine	6.38	7.46	9.40	8.88	0.99	0.781	0.040	0.432

Table 5. Mean concentrations (n = 5) of VFA [mmol/l] after 6 and 24 h of an *in vitro* incubation of feline faecal inoculum with a control diet to which different substrates were added.

	Group CTRL	Group CT	Group YSE	Group CT + YSE	Pooled SEM	ANOVA *p*-value		
						Group CT	Group YSE	Group CT + YSE
At 6 h								
Acetic acid	14.8	13.3	12.7	12.1	0.34	0.082	0.010	0.385
Propionic acid	7.09	6.51	7.16	5.99	0.18	0.010	0.446	0.335
Isobutyric acid	0.53	0.39	0.51	0.53	0.05	0.583	0.602	0.498
n-Butyric acid	7.54	7.96	7.58	7.49	0.16	0.666	0.558	0.489
Isovaleric acid	0.63	0.65	0.62	0.68	0.04	0.646	0.894	0.788
n-Valeric acid	0.38	0.32	0.25	0.24	0.02	0.348	0.006	0.455
Total VFA	31.0	29.1	28.8	27.1	0.55	0.092	0.052	0.942
At 24 h								
Acetic acid	24.2	22.4	23.6	21.7	0.44	0.044	0.451	0.961
Propionic acid	12.7	10.6	12.8	10.0	0.34	<0.0001	0.681	0.393
Isobutyric acid	1.21	1.21	1.11	1.10	0.05	0.974	0.383	0.987
n-Butyric acid	10.1	9.68	9.86	9.29	0.14	0.095	0.292	0.718
Isovaleric acid	2.01	1.95	2.26	1.80	0.07	0.039	0.686	0.108
n-Valeric acid	2.29	3.03	2.60	3.12	0.19	0.109	0.599	0.774
Total VFA	52.5	48.9	52.3	47.1	0.75	0.002	0.422	0.497

Table 6. Microbial populations [log 10 DNA copies/ml] detected by qPCR in feline faecal inoculum with a control diet to which different substrates were added. Values are the means of 5 bottles per treatment.

	Group CTRL	Group CT	Group YSE	Group CT + YSE	Pooled SEM	ANOVA *p*-value		
						Group CT	Group YSE	Group CT + YSE
At 6 h								
Lactobacillus spp.	8.63	7.67	7.87	7.83	0.17	0.153	0.368	0.181
Enterococcus spp.	6.13	7.82	9.42	3.87	0.73	0.105	0.769	0.008
Bifidobacterium spp.	2.70	1.51	1.09	1.60	0.28	0.154	0.160	0.119
E. coli	8.26	7.21	7.00	7.29	0.21	0.311	0.132	0.092
At 24 h								
Lactobacillus spp.	7.18	6.46	6.65	6.27	0.17	0.122	0.294	0.611
Enterococcus spp.	7.53	7.84	5.66	7.64	0.48	0.245	0.290	0.388
Bifidobacterium spp.	2.39	1.39	1.46	1.16	0.26	0.228	0.278	0.512
E. coli	6.32	5.53	5.62	5.32	0.23	0.259	0.335	0.603

Figure 1. PCA score plot of pH, bacterial metabolites and microbial populations on canine faecal inoculum

Figure 2. PCA score plot of pH, bacterial metabolites and microbial populations on feline faecal inoculum